Amendments to the Specification:

Please replace the title with the following amended title:

-- ANTI-ENDOTOXIC, ANTIMICROBIAL CATIONIC PEPTIDES, THE ENCODING DNA AND METHODS OF USE THEREOF. --

Please replace paragraph [0001] with the following amended paragraph:

[0001] This application is a divisional application of U.S. Serial No. 09/908,139 filed July 17, 2001, now ~~pending~~ issued as U.S. Patent No. 6,818,407; which is a divisional application of U.S. Application Serial No. 09/143,124 filed August 28, 1998, now issued as U.S. ~~patent~~ Patent No. 6,288,212. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application

Please replace paragraph [0003] with the following amended paragraph:

[0003] It has been clearly demonstrated that the outer membranes of Gram-negative bacteria are semipermeable molecular ~~Asieves≅~~ "sieves" which restrict access of antibiotics and host defense molecules to their targets within the bacterial cell. Thus, cations and polycations which access the self-promoted uptake system are, by virtue of their ability to interact with and break down the outer membrane permeability barrier, capable of increasing the susceptibility of Gram-negative pathogenic bacteria to antibiotics and host defense molecules. Hancock and Wong demonstrated that a broad range of such compounds could overcome the permeability barrier and coined the name "permeabilizers" to describe them (Hancock and Wong, *Antimicrob. Agents Chemother.*, 26:48, 1984). While self-promoted uptake and permeabilizers were first described for *P. aeruginosa*, they have now been described for a variety of Gram-negative bacteria.

Please replace paragraph [0022] with the following amended paragraph:

[0022] Figure 1 is a graph which shows LPS (endotoxin) binding by the dansyl polymyxin binding assay. The anti-endotoxic activity of the peptides was tested in the murine cell line

RAW 264.7 which was obtained from the ATCC (~~Rockville, MD~~ Manassas, VA). ~~Symbols in the graph are as follows: ΣΣ ΣΣ 28; ΣΣMΣΣ CM1; ΣΣΣ‹›ΣΣ CM2; ΣΣ∀ ΣΣ CM3; ΣΣPΣΣ CM4; ΣΣ ΣΣ CM5; ΣΣ ΣΣ CM6; ΣΣ9ΣΣ CM7.~~

Please replace paragraph [0026] with the following amended paragraph:

[0026] The term ~~Aantimicroial≅~~ "antimicrobial" as used herein means that the peptides of the present invention inhibit, prevent, or destroy the growth or proliferation of microbes such as bacteria, fungi, viruses, parasites or the like. The term ~~Aantiviral≅~~ "antiviral" as used herein means that the peptides of the present invention inhibit, prevent or destroy the growth or proliferation of viruses or of virally-infected cells. The term ~~Aanti-tumor≅~~ "anti-tumor" as used herein means that the peptides of the present invention may be used to inhibit the growth of tumor cells. The term ~~Aantifungal≅~~ "antifungal" as used herein means that the peptides of the present invention may be used to inhibit the growth of or destroy fungi. The term ~~Aantiparasite≅,~~ "antiparasite," as used herein, means that the peptides of the present invention inhibit, prevent, or destroy the growth or proliferation of any organism that lives at the expense of a host organism.

Please replace paragraph [0028] with the following amended paragraph:

[0028] The term ~~Aisolated≅~~ "isolated" as used herein refers to a peptide substantially free of proteins, lipids, nucleic acids, for example, with which it is naturally associated. Those of skill in the art can make similar substitutions to achieve peptides with greater antimicrobial activity and a broader host range. For example, the invention includes the peptides depicted in SEQ ID NOs:3-12 and 14-21, as well as analogues, derivatives and amidated derivatives thereof, as long as a bioactivity (e.g., antimicrobial) of the peptide remains. Minor modifications of the primary amino acid sequence of the peptides of the invention may result in peptides which have substantially equivalent or enhanced activity as compared to the specific peptides described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the peptides produced by these modifications are included herein as long as the biological activity of the original peptide still exists or, in the case of amidated versions of

the peptide, the antimicrobial activity of the original peptide is enhanced or altered such that the amidated peptide is therapeutically useful. For example, the amino acid sequence of SEQ ID NO:16 is identical to that of SEQ ID NO:15. However, SEQ ID NO:16 is amidated at the C-terminal end thereby altering the antimicrobial activity of the peptide. It is envisioned that such modifications are useful for altering or enhancing the biological activity of a particular peptide.

Please replace paragraph [0030] with the following amended paragraph:

[0030] A ~~Apeptide≅~~ "peptide" of the invention includes amino acid sequences are conservative variations of those peptides specifically exemplified herein. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substituted for one another include asparagine, glutamine, serine and threonine. The term ~~Aconservative variation≅~~ "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Such conservative substitutions are within the definition of the classes of the peptides of the invention.

Please replace paragraph [0031] with the following amended paragraph:

[0031] The biological activity of the peptides can be determined by standard methods known to those of skill in the art, such as ~~Aminimal inhibitory concentration≅~~ "minimal inhibitory concentration" (MIC) assay described in the present examples, whereby the lowest concentration at which no change in OD is observed for a given period of time is recorded as MIC. Alternatively, ~~Afractional inhibitory concentration≅~~ "fractional inhibitory concentration" (FIC) is also useful for determination of synergy between the peptides of the invention, or the peptides in combination with known antibiotics. FICs are performed by checkerboard titrations of

peptides in one dimension of a microtiter plate, and of antibiotics in the other dimension, for example. The FIC is calculated by looking at the impact of one antibiotic on the MIC of the other and vice versa. An FIC of one indicates that the influence of the compounds is additive and an FIC of less than one indicates synergy. Preferably, an FIC of less than 0.5 is obtained for synergism. As used herein, FIC can be determined as follows:

$$\text{~~FIG~~ FIC} = \frac{\text{MIC (peptide in combination)}}{\text{MIC (peptide alone)}} + \frac{\text{MIC (antibiotic in combination)}}{\text{MIC (antibiotic alone)}}$$

Please replace paragraph [0034] with the following amended paragraph:

[0034] The term ~~Aisolated≅~~ "isolated" as used herein refers to a polynucleotide substantially free of proteins, lipids, nucleic acids, for example, with which it is naturally associated. As used herein, ~~Apolypeptide≅~~ "polynucleotide" refers to a polymer of deoxyribonucleotides or ribonucleotides, in the form of a separate fragment or as a component of a larger construct. DNA encoding a peptide of the invention can be assembled from cDNA fragments or from oligonucleotides which provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit. Polynucleotide sequences of the invention include DNA, RNA and cDNA sequences. A polynucleotide sequence can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. Polynucleotides of the invention include sequences which are degenerate as a result of the genetic code. Such polynucleotides are useful for the recombinant production of large quantities of a peptide of interest, such as the peptide of SEQ ID NOs:3-21.

Please replace paragraph [0043] with the following amended paragraph:

[0043] The term ~~Acontacting≅~~ "contacting" refers to exposing the bacteria to the peptide so that the peptide can effectively inhibit, kill, or lyse bacteria, bind endotoxin (LPS), or permeabilize gram-negative bacterial outer membranes, for example. Contacting may be *in vitro*, for example by adding the peptide to a bacterial culture to test for susceptibility of the bacteria to the peptide. Contacting may be *in vivo*, for example administering the peptide to a subject with a bacterial disorder, such as septic shock. ~~Ainhibiting≅~~ "inhibiting" or ~~Ainhibiting~~

~~effective amount~~ "inhibiting effective amount" refers to the amount of peptide which is required to cause a bacteriostatic or bactericidal effect. Examples of bacteria which may be inhibited include *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus typhimurium*, *Staphylococcus aureus*, *Enterobacter facaelis*, *Listeria monocytogenes*, *Corynebacterium xerosis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus mitis*, *Staphylococcus epidermidis* and *Staphylococcus aureus* K147.

Please replace paragraph [0049] with the following amended paragraph:

[0049] Preparations for parenteral administration of a peptide of the invention include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, ~~ARinger=s~~ "Ringer's" dextrose, dextrose and sodium chloride, lactated ~~ARinger=s,~~ "Ringer's," or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Please replace paragraph [0058] with the following amended paragraph:

[0058] The invention also provides a method of treating or ameliorating an endotoxemia or septic shock (sepsis) associated disorder, or one or more of the symptoms of sepsis comprising administering to a subject displaying symptoms of sepsis or at risk for developing sepsis, a therapeutically effective amount of a cationic peptide of the invention, for example, SEQ ID NOs:3-12, or analogs, derivatives, amidated variations or conservative variations thereof. The term ~~Aameliorate=~~ "ameliorate" refers to a decrease or lessening of the symptoms of the disorder being treated. Such symptoms which may be ameliorated include those associated with a transient increase in the blood level of TNF, such as fever, hypotension, neutropenia, leukopenia, thrombocytopenia, disseminated intravascular coagulation, adult respiratory distress syndrome, shock and multiple organ failure. Patients who require such treatment include those

at risk for or those suffering from toxemia, such as endotoxemia resulting from a gram-negative bacterial infection, venom poisoning, or hepatic failure, for example. In addition, patients having a gram-positive bacterial, viral or fungal infection may display symptoms of sepsis and may benefit from such a therapeutic method as described herein. Those patients who are more particularly able to benefit from the method of the invention are those suffering from infection by *Escherichia coli*, *Haemophilus influenza B*, *Neisseria meningitides*, staphylococci, or pneumococci. Patients at risk for sepsis include those suffering from gunshot wounds, renal or hepatic failure, trauma, burns, immunocompromised (HIV), hematopoietic neoplasias, multiple myeloma, Castleman's disease or cardiac myxoma. Methods for producing antimicrobial peptides of the invention effective for treating endotoxin-associated disorders are set forth in PCT Application Serial Number PCT/CA93/00342, incorporated herein by reference in its entirety.

Please replace paragraph [0059] with the following amended paragraph:

[0059] The term ~~Atherapeutically effective amount≅~~ "therapeutically effective amount" as used herein for treatment of endotoxemia refers to the amount of cationic peptide used is of sufficient quantity to decrease the subject's response to LPS and decrease the symptoms of sepsis. The term ~~Atherapeutically effective≅~~ "therapeutically effective" therefore includes that the amount of cationic peptide sufficient to prevent, and preferably reduce by at least 50%, and more preferably sufficient to reduce by 80%, a clinically significant increase in the plasma level of TNF. The dosage ranges for the administration of cationic peptide are those large enough to produce the desired effect. Generally, the dosage will vary with the age, condition, sex, and extent of the infection with bacteria or other agent as described above, in the patient and can be determined by one skilled in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the level of LPS and TNF in a patient. A decrease in serum LPS and TNF levels should correlate with recovery of the patient.

Please replace paragraph [0062] with the following amended paragraph:

[0062] The term ~~Abactericidal amount≅~~ "bactericidal amount" as used herein refers to an amount sufficient to achieve a bacteria-killing blood concentration in the patient receiving the treatment. The bactericidal amount of antibiotic generally recognized as safe for administration to a human is well known in the art, and as is known in the art, vanes with the specific antibiotic and the type of bacterial infection being treated.

Please replace paragraph [0066] with the following amended paragraph:

[0066] As used herein, a ~~Atherapeutically effective amount≅~~ "therapeutically effective amount" of a composition containing an antimicrobial peptide of the invention with or without an active biologic agent means that which stimulates or induces cell growth. While not wanting to be bound to a particular theory, a therapeutically effective amount is beneficial for augmenting tissue repair by promoting tissue regeneration while simultaneously inhibiting or preventing pathogenic microbial growth. Diseases, infections, disorders or ailments benefitting from such modulation of tissue growth and inhibition of pathogenic microbial growth include, but are not limited to, tissue repair subsequent to traumatic injuries, conditions including arthritis, osteoporosis and other skeletal disorders, damage due to chronic bronchitis, damage due to smoke inhalation, damage due to a host immune response, damage due to fungal, bacterial, viral, protozoan, and parasitic diseases, and burns, for example. Because these problems are likely due to a poor growth response of the fibroblasts, stem cells, chondrocytes, osteoblasts or fibroblasts at the site of injury, the addition of an active biologic agent that stimulates or induces growth of these cells is beneficial. The term ~~Ainduce≅~~ "induce" or ~~Ainduction≅~~ "induction" as used herein, refers to the activation, stimulation, enhancement, initiation and or maintenance of the cellular mechanisms or processes necessary for the formation of any of the tissue, repair process or development as described herein.

Please replace paragraph [0067] with the following amended paragraph:

[0067] In another aspect, the invention is useful for revitalizing scar tissue resulting from microbial (e.g., fungal, parasitic, viral infection, bacterial infection or protozoan infections) injuries due to surgical procedures, irradiation, laceration, toxic chemicals or burns, for example. The term ~~Ascar tissue≅~~ "scar tissue" means fibrotic or collagenous tissue formed during the

healing of a wound. For example, antimicrobial peptides can be included in a controlled release matrix which can be positioned in proximity to damaged tissue thereby promoting regeneration and revascularization of such tissue. The term ~~Acontrolled release matrix≅~~ "controlled release matrix" means any composition which allows the slow release of a bioactive substance which is mixed or admixed therein. The matrix can be a solid composition, a porous material, or a semi-solid, gel or liquid suspension containing bioactive substances. The term ~~Abioactive material≅~~ "bioactive material" means any composition that will modulate tissue repair when used in accordance with the method of the present invention. The bioactive materials/matrix can be introduced by means of injection, surgery, catheters or any other means suitable for modulating tissue repair.

Please replace paragraph [0089] with the following amended paragraph:

[0089] CD-1 mice were induced to be neutropenic via 3 intraperitoneal injections of cyclophosphamide (150 ~~□g/kg/per~~ µg/kg per injection) every another day. Immediately after the third administration of cyclophosphamide, the mice were challenged by intraperitoneal injection of *Pseudomonas aeruginosa* strain M2 (200 - 300 organisms/mouse). Cationic peptides (200 ~~□g~~ µg per mouse = 8 mg/kg) in 100 ~~□l~~ µl buffered citrate were injected intraperitoneally at 30 min (single dose) or 30 min and 16 hrs (double dose) post bacterial challenge, respectively. The data were the average values of 2 individual experiments. PBS was used as a control. The bolded columns demonstrate better peptide protection than the CP26 and CP29 (SEQ ID NO:25 and SEQ ID NO:2, respectively) controls. In general single dose protection studies gave better protection than double dose experiments. Protection in non-neutropenic mice was not as impressive but the same peptides showed as good or better killing than SEQ ID NO:25.

Please replace paragraph [0094] with the following amended paragraph:

[0094] Constant delivery of peptide P-CN using intraperitoneal mini-osmotic pumps was carried out. Briefly, juvenile coho salmon were divided into three treatment groups: A. Bacterial injection alone (12 fish). B. Fish saline osmotic pump and bacterial injection (12 fish). C. A combination of P-CN osmotic pump and bacterial injection (19 fish). The fish were anesthetized and implanted (peritoneal cavity) with mini-osmotic pumps having a pumping rate

of 0.13 ~~□1/hour~~ µl/hour. Heaters were placed in the tanks to keep the water temperature between 12 and 13°C. Pumps were filled with concentrated P-CN to deliver approximately 250 µl/day peptide to fish over a 30-day period. Twelve days after pump implantation, the fish received intraperitoneal injections of *V. anguillarum* (105 bacteria/fish). Mortalities were recorded daily and are shown in Table 12. Mortalities were first noticed on day 3 for the group injected with bacteria alone and on day 5 for the group which received saline osmotic pumps as well as bacterial injections. However, there was no significant difference in mortality between the bacterial injection alone group and the saline osmotic pump group (67% vs. 75%). Mortalities were delayed for the P-CN osmotic pump group. The P-CN osmotic pump group had only one fish die on day 6 over the 30 days experiments with an accumulated mortality of 5%. These results suggested that P-CN was very effective in delaying and reducing mortality in *V. anguillarum* infected fish. Furthermore, since cationic peptides are not effective in a single treatment and constant administration is necessary, this argues for the potential success of transgenic fish expressing peptide P-CN.

Please replace paragraph [0096] with the following amended paragraph:

[0096] The propeptide region and antimicrobial peptide are fused to the construct containing combination of the promoter, signal peptide, and terminator using standard PCR procedures. Four primers are designed for PCR. Primer ~~□a□~~ "a" includes several ~~3□~~ 3' sequences of GH 1-SP and ~~5□~~ 5' sequences of the propeptide region. Primer ~~□b□~~ "b" contains ~~3□~~ 3' sequences of the pro region and ~~5□~~ 5' sequences of antimicrobial peptide gene. Primer ~~□c□~~ "c" includes the ~~3□~~ 3' end of the pro region and ~~5□~~ 5' end of the antimicrobial peptide gene. Primer ~~□d□~~ "d" is a combination of the ~~3□~~ 3' end of the antimicrobial peptide gene and an XbaI site. The complete construct is then cloned into ~~thepBluescript□~~ the pBluescript® II KS plasmid and the construct DNA was thus generated. Prior to gene transfer all the vector sequences were removed by cleavage at *NotI* sites.